

**This is an electronic reprint of the original article.  
This reprint *may differ* from the original in pagination and typographic detail.**

**Author(s):** Kahilainen, Aapo; Keränen, Inka; Kuitunen, Katja; Kotiaho, Janne Sakari; Knott, Emily

**Title:** Interspecific interactions influence contrasting spatial genetic structures in two closely related damselfly species

**Year:** 2014

**Version:**

**Please cite the original version:**

Kahilainen, A., Keränen, I., Kuitunen, K., Kotiaho, J. S., & Knott, E. (2014). Interspecific interactions influence contrasting spatial genetic structures in two closely related damselfly species. *Molecular Ecology*, 23(20), 4976-4988.  
<https://doi.org/10.1111/mec.12916>

All material supplied via JYX is protected by copyright and other intellectual property rights, and duplication or sale of all or part of any of the repository collections is not permitted, except that material may be duplicated by you for your research use or educational purposes in electronic or print form. You must obtain permission for any other use. Electronic or print copies may not be offered, whether for sale or otherwise to anyone who is not an authorised user.

**Interspecific interactions influence contrasting spatial genetic structures in two closely related damselfly species**

Journal:	<i>Molecular Ecology</i>
Manuscript ID:	MEC-14-0691.R1
Manuscript Type:	Original Article
Date Submitted by the Author:	n/a
Complete List of Authors:	Kahilainen, Aapo; University of Jyväskylä, Department of Biological and Environmental Science Keränen, Inka; University of Jyväskylä, Department of Biological and Environmental Science Kuitunen, Katja; University of Jyväskylä, Department of Biological and Environmental Science; University of Jyväskylä, Department of Mathematical Information Technology Kotiaho, Janne; University of Jyväskylä, Department of Biological and Environmental Science; University of Jyväskylä, Natural History Museum Knott, Karelyn; University of Jyväskylä, Department of Biological and Environmental Science
Keywords:	Calopteryx splendens, Calopteryx virgo, population genetics, Landscape Genetics, microsatellite

1 Title: **Interspecific interactions influence contrasting spatial genetic**  
2 **structures in two closely related damselfly species**

3 Authors: Aapo Kahilainen<sup>1</sup>, Inka Keränen<sup>1</sup>, Katja Kuitunen<sup>1,2</sup>, Janne S.  
4 Kotiaho<sup>1,3</sup> & K. Emily Knott<sup>1</sup>

5 Affiliations: <sup>1</sup>*University of Jyväskylä, Department of Biological and*  
6 *Environmental Science,*  
7 *P.O. Box 35, FI-40014, University of Jyväskylä, Finland*

8 <sup>2</sup>*University of Jyväskylä, Department of Mathematical Information*  
9 *Technology,*  
10 *P.O.Box 35, FI-40014 University of Jyväskylä, Finland.*

11 <sup>3</sup>*University of Jyväskylä, Natural History Museum,*  
12 *P.O. Box 35 FI-40014, University of Jyväskylä, Finland*

13 Keywords: *Calopteryx splendens, Calopteryx virgo, population genetics,*  
14 *landscape genetics, microsatellite*

15 Correspondence: Aapo Kahilainen  
16 University of Jyväskylä, Department of Biological and  
17 Environmental Science,  
18 P.O. Box 35, FI-40014, University of Jyväskylä, Finland  
19 Tel: +358 40 8053875; Fax: +358 14 617 239  
20 E-mail: [aapo.kahilainen@jyu.fi](mailto:aapo.kahilainen@jyu.fi)

21 Running title: *Contrasting genetic structures in Calopteryx*

22 *ABSTRACT*

23 Spatial genetic structure (SGS) is largely determined by colonization history, landscape and  
24 ecological characteristics of the species. Therefore, sympatric and ecologically similar species  
25 are expected to exhibit similar SGSs, potentially enabling prediction of the SGS of one  
26 species from that of another. On the other hand, due to interspecific interactions, ecologically  
27 similar species could have different SGSs. We explored the SGSs of the closely related  
28 *Calopteryx splendens* and *C. virgo* within Finland and related the genetic patterns to  
29 characteristics of the sampling localities. We observed different SGSs for the two species.  
30 Genetic differentiation even within short distances in *C. splendens* suggests genetic drift as an  
31 important driver. However, we also observed indication of previous gene flow (revealed by a  
32 negative relationship between genetic differentiation and increasing potential connectivity of  
33 the landscape). Interestingly, genetic diversity of *C. splendens* was negatively related to  
34 density of *C. virgo*, suggesting that interspecific interactions influence the SGS of *C.*  
35 *splendens*. In contrast, genetic differentiation between *C. virgo* sub-populations was low and  
36 only exhibited relationships with latitude, pointing to high gene flow, colonization history and  
37 range margin effects as the drivers of SGS. The different SGSs of the two ecologically similar  
38 species cautions indirect inferences of SGS based on ecologically similar surrogate species.

39 *INTRODUCTION*

40 The spatial distribution of genetic diversity is rarely homogenous, but exhibits pronounced  
41 geographic variation (Eckert *et al.* 2008). The distribution of genetic diversity within and  
42 between populations, i.e. spatial genetic structure (SGS), is driven by historic contingencies  
43 such as range contractions and expansions due to glacial cycles (Hewitt 2000) and  
44 contemporary processes of random genetic drift (Kimura & Crow 1964), gene flow between  
45 populations (Slatkin 1985; Bohonak 1999), and selection (Ranta *et al.* 2009; Funk *et al.*  
46 2011). Since these drivers of SGS are strongly influenced by the ecology of the species (e.g.  
47 habitat preferences, dispersal abilities, mating system and life-history characteristics) and by  
48 environmental characteristics of the landscape (e.g. the amount of habitat suitable for  
49 population persistence and dispersal), sympatric and ecologically similar species can be  
50 expected to exhibit similar SGSs (Whiteley *et al.* 2006; Fortuna *et al.* 2009; Dawson 2012;  
51 Hughes *et al.* 2013).

52 On the other hand, increasing ecological similarity of species can lead to increasing  
53 interspecific interactions, and thus affect their SGSs. For example, in a simulation, increasing  
54 the strength of interspecific competition and the number of competing species decreased both  
55 effective population sizes and dispersal between localities, leading to increased genetic drift  
56 and differentiation between populations (Ranta *et al.* 2009). Similarly, increasing the number  
57 of competitor species led to decreased population sizes and increased genetic drift of  
58 experimental perennial ryegrass (*Lolium perenne*) populations (Nestmann *et al.* 2011).

59 Given that the SGS of species is important for determining management units and predicting  
60 the outcomes of land use on the viability and evolutionary potential of populations (Frankham

61 2005; Whiteley *et al.* 2006; Fortuna *et al.* 2009) the generalizability of SGS across sympatric  
62 and ecologically similar species is important also for applied conservation projects. If SGSs  
63 can be generalized across species, SGSs can be predicted for other sympatric and ecologically  
64 similar species based on that of only one (or a few) representative species. This implies that  
65 conservation strategies designed for safeguarding the viability and evolutionary potential of a  
66 single species could be effective in conserving other species as well (Whiteley *et al.* 2006).  
67 On the other hand, if interspecific interactions between the focal species lead to differences in  
68 their SGSs, conservation strategies targeted for single species systems might not be adequate.  
69 In these cases the SGSs of each species of conservation concern would need to be determined  
70 separately.

71 We studied the SGSs of *Calopteryx splendens* [HARRIS, 1782] and *C. virgo* [LINNAEUS,  
72 1758] in Finland (Fig. 1). The two damselfly species provide a suitable system for studying  
73 the generalizability of SGS across species with largely similar ecologies, since they are  
74 considerably similar in their appearance, behavior, life-history and habitat choice (Askew  
75 2004; Wellenreuther *et al.* 2012; Karjalainen & Hämäläinen 2013; but see Sternberg &  
76 Buchwald 1999). To fully describe the SGSs of both species we determined how genetic  
77 variation is distributed within (allelic richness and heterozygosity) and between sub-  
78 populations (isolation-by-distance, population specific  $F_{ST}$ s, pairwise  $F_{ST}$ s and  $D_{est}$ s, and  
79 number of genetic clusters). From each sub-population, we also recorded locality  
80 characteristics (latitude and longitude, density of conspecific males and connectivity of the  
81 landscape), which we considered to be associated with colonization history, random genetic  
82 drift and dispersal between localities. To estimate the frequency of interspecific interactions  
83 experienced, we recorded the density of heterospecific *Calopteryx* males at each sub-  
84 population. Our aims were to see (1) if the SGSs of the two *Calopteryx* species are

85 concordant, (2) if the SGSs of the species have similar relationships with the characteristics of  
86 the localities, and (3) if the SGSs of the two species are affected by interspecific interactions.

## 87 *METHODS*

### 88 ***Calopteryx* species**

89 The Eurasian *C. splendens* and *C. virgo* have largely overlapping biogeographic ranges with  
90 both species inhabiting small rivers and streams across most of Europe (Askew 2004;  
91 Karjalainen & Hämäläinen 2013). Within their sympatric range in southern Fennoscandia, the  
92 environmental niches of the *Calopteryx* species also overlap, with the niche of *C. splendens*  
93 being nested within that of *C. virgo* (Wellenreuther *et al.* 2012). However, some habitat  
94 partitioning between the two species has been reported in Central Europe, where *C. splendens*  
95 is most often found in slightly warmer (18-24°C) lowland rivers and *C. virgo* is found in  
96 cooler and more shaded rivers (13-18°C) at higher elevations (Sternberg & Buchwald 1999;  
97 Schütte & Schrimpf 2002). The different temperature affinities of the two species are  
98 reflected in differences in their range limits in the north: the range of *C. splendens* extends to  
99 roughly 64° Lat., whereas the range of *C. virgo* extends to 68° Lat. (Valle 1952; Karjalainen  
100 2010; Karjalainen & Hämäläinen 2013). However, although *C. virgo* seems to occupy a wider  
101 range of water temperatures, we found no habitat partitioning with respect to water  
102 temperature in the two *Calopteryx* species at our study sites (Supplementary material A).  
103 Also, since the wings of *C. virgo* are more melanised than those of *C. splendens*, it has been  
104 suggested that *C. virgo* has better thermoregulation abilities allowing them to adapt to colder  
105 environments (Outomuro & Ocharan 2011). However, recent work concluded that differences

106 in wing melanisation between the *Calopteryx* species is primarily driven by sexual selection,  
107 and that it is less important for adaptation to colder environments (Svensson & Waller 2013).

108 Capture-mark-recapture studies indicate that sexually mature individuals of both *Calopteryx*  
109 species rarely disperse more than 300 meters from the site of first observation (Stettmer 1996;  
110 Schutte *et al.* 1997; Ward & Mill 2007). The longest observed dispersal distances of adult  
111 *Calopteryx* individuals have been 1.7 km and 4 km for *C. splendens* and *C. virgo*, respectively  
112 (Stettmer 1996). We are not aware of studies explicitly focusing on dispersal prior to sexual  
113 maturation (i.e. natal dispersal), but we expect it to be limited since significant population  
114 differentiation has been reported for *C. splendens* (overall  $F_{ST}$ s range from 0.05 to 0.14 in  
115 different studies; Svensson *et al.* 2004; Chaput-Bardy *et al.* 2008; Viitaniemi 2009). However,  
116 we are not aware of any study on the SGS of *C. virgo*.

117 In Finland, *C. splendens* sub-populations are nearly exclusively sympatric with *C. virgo*,  
118 although *C. virgo* sub-populations can be locally allopatric within the distribution range of *C.*  
119 *splendens* (Tynkkynen *et al.* 2004; but see Ilvonen *et al.* 2011). The species frequently  
120 interact in locally sympatric sub-populations and occasionally hybridize (Svensson *et al.*  
121 2007; Keränen *et al.* 2013). Both species are territorial, and the males exhibit interspecific  
122 aggression when competing for breeding territories (De Marchi 1990; Tynkkynen *et al.* 2004,  
123 2006). *C. virgo* seems to be the stronger competitor (Tynkkynen *et al.* 2004, 2006), and  
124 interspecific interactions with *C. virgo* are known to influence the secondary sexual characters  
125 of *C. splendens* (Tynkkynen *et al.* 2005; Honkavaara *et al.* 2011; Kuitunen *et al.* 2011). Since  
126 the mating success of *Calopteryx* males is related to their territory holding ability (Plaistow &  
127 Siva-Jothy 1996), interspecific competition could reduce the effective population size of the  
128 weaker competitor. Indeed, a previous study suggested that the genetic diversity of *C.*



129 *splendens* declines as the proportion of *C. virgo* individuals in sympatric sub-populations  
130 increases (Viitaniemi 2009).

### 131 **Sampling localities and collecting *Calopteryx* individuals**

132 Sampling was conducted between 27<sup>th</sup> of June and 29<sup>th</sup> of July, and between 23<sup>rd</sup> of June and  
133 28<sup>th</sup> of July, in 2008 and 2009, respectively. Nineteen of 40 sampling localities contained both  
134 *C. splendens* and *C. virgo* (i.e. sympatric sub-populations) and the others contained only *C.*  
135 *virgo* (i.e. allopatric sub-populations), nine of which were located in Northern Finland,  
136 outside the range of *C. splendens* (Fig. 1). Considering the relatively poor dispersal  
137 capabilities of *Calopteryx* damselflies (see above), we decided to sample sub-populations  
138 from different rivers separated by at least one lake and a minimum distance of ten kilometers,  
139 an exception being *C. virgo* sub-populations AJ and AK (Fig. 1; Supplementary material B,  
140 Table B1), which were only 4.9 km apart.

141 *Calopteryx* individuals were collected using butterfly nets. Either whole individuals or a  
142 single leg per individual (if population sizes seemed low) were stored in 95% ethanol (EtOH)  
143 at 6-8°C until DNA extraction. Removing a leg from damselflies does not impact fitness  
144 (Thompson *et al.* 2011). Since only two *C. splendens* individuals were collected from sub-  
145 population BG, these individuals were not genotyped or included in the analyses. Therefore,  
146 the sample size for *C. splendens* sub-populations was 18. For most sub-populations,  
147 genotyped individuals were collected in either 2008 or 2009, but for populations AA, AC, AD  
148 and AK (Supplementary material B, Table B1.) we genotyped individuals collected in both  
149 years to test for temporal population structure (Supplementary material C).

## 150 **Recording locality characteristics**

151 Geographical coordinates were recorded on site with a hand-held GPS device (Garmin eTrex  
152 Legend HCx or Magellan Triton 300). Latitude and longitude were used in our analyses as  
153 UTM coordinates according to grid ETRS-TM35FIN. *Calopteryx* densities were estimated by  
154 counting all individuals within a 300-meter section of river, which was defined as 150 meters  
155 both up- and downstream from the site of the perceived highest density of *Calopteryx*  
156 individuals. Although both male and female density were estimated, only male densities were  
157 included in the analyses, since females frequently move away from rivers in response to  
158 changing weather, making estimates of their density less reliable (K. Kuitunen, pers. obs.).  
159 All counts were recorded between 9 AM and 4 PM, when the damselflies are most active  
160 (Corbet 1999; Karjalainen & Hämäläinen 2013). Densities at most sites were estimated at  
161 least twice during the season and some were also estimated in both years (Supplementary  
162 material B, Table B1). Repeatability of the density estimates was high (*C. splendens*:  
163 repeatability=0.64,  $F=5.745$ ,  $df_1=16$ ,  $df_2=29$ ,  $n_0=2.63$ ; *C. virgo*: repeatability=0.62,  $F=5.477$ ,  
164  $df_1=26$ ,  $df_2=47$ ,  $n_0=2.77$ ; Lessells & Boag 1987). We used logarithm-transformed ( $\log_{10}$ )  
165 averages of the multiple estimates in our analyses. In the case of *C. splendens*, we added one  
166 to each density estimate before transformation, since several sites did not contain a single *C.*  
167 *splendens* individual. For simplicity, the logarithm-transformed density estimates are  
168 hereafter referred to simply as density estimates.

169 To obtain a locality specific measure for potential connectivity we quantified the total length  
170 of river habitat within a five-kilometer radius buffer zone from each sampling site. Width of  
171 river habitat was not included because breeding sites of *Calopteryx* are mainly found along  
172 the shoreline. Measurements were made from a combination of two map layers (“River

173 Network” & “Water formations, EU Water Framework Directive”, provided by The Finnish  
174 Environment Institute & Centres for Economic Development, Transport and the  
175 Environment) using ArcGIS 10.1 (ESRI 2012. ArcGIS Desktop: Release 10. Redlands, CA:  
176 Environmental Systems Research Institute). Our measure reflects potential connectivity  
177 because it does not consider whether the habitat contains *Calopteryx* individuals (Calabrese &  
178 Fagan 2004; Hughes *et al.* 2013). Our measure neglects the potentially important branching  
179 structure of the rivers (Chaput-Bardy *et al.* 2008), however, it more accurately reflects our  
180 sampling scheme, given that we sampled sub-populations residing in different rivers rather  
181 than different tributaries of the same river. The five kilometer radius buffer should sufficiently  
182 represent potential connectivity since this distance is at the higher range of dispersal for both  
183 *C. virgo* and *C. splendens* (Stettmer 1996; Schutte *et al.* 1997).

184 Prior to incorporating any of the variables into the analyses we checked the data for  
185 collinearity and spatial autocorrelation (Moran’s I). Collinearity was tested by calculating  
186 variance inflation factors (VIF) for all variables in R (R Core Team 2012), and spatial  
187 autocorrelation was tested across the entire 40 site dataset using SAM 4.0 (Rangel *et al.*  
188 2010). VIFs were low for all variables (all below 2), suggesting no collinearity. However, a  
189 negative spatial autocorrelation (Moran’s I = -0.321; P=0.005) was observed in the density of  
190 *C. splendens* males in the longest distance class (center at c.a. 607 km). This occurred because  
191 nine of the sampled localities (*C. virgo* sub-populations) were outside the distribution range of  
192 *C. splendens*, resulting in spatial aggregation of localities with zero density of *C. splendens*  
193 (Fig. 1). Thus, we analyzed a separate subset of the *C. virgo* data containing only the  
194 sympatric populations (see below). There was no spatial autocorrelation in any of the other  
195 variables.

## 196 **Population Genetic Methods**

197 DNA was extracted from the legs of *Calopteryx* individuals and used for genotyping of  
198 **microsatellite loci** as described in Supplementary material C and Molecular Ecology  
199 Resources Primer Development Consortium et al. (2011). The microsatellite loci were  
200 assessed for reliable scoring and potential deviations from equilibrium and neutrality  
201 assumptions (Supplementary material C). Finally, the *C. splendens* dataset included 567  
202 individuals genotyped at 12 loci and the *C. virgo* dataset included 1401 individuals genotyped  
203 at six loci (Supplementary material C). For each locus and subpopulation, unbiased expected  
204 heterozygosity ( $uH_E$ ) estimated using GenAlEx 6.5 (Peakall & Smouse 2012) and allelic  
205 richness ( $AR$ ) adjusted by rarefaction was estimated using FSTAT 2.9.3 (updated from  
206 Goudet 1995; See Supplementary material C, Tables C3 and C4).

## 207 **Statistical Methods**

208 We studied how environmental characteristics (see above) and density of heterospecific males  
209 are related to intrapopulation genetic diversity as measured by  $uH_E$  and  $AR$ , using a beta  
210 regression and a linear multiple regression, respectively. Analyses were conducted using R  
211 and the package “betareg” (Cribari-Neto & Zeileis 2010) was used for the beta regression. All  
212 independent variables were initially entered into the model and then removed on a stepwise  
213 basis by comparing nested models with a log-likelihood ratio test.

214 To study how genetic diversity was distributed among populations (i.e. population  
215 differentiation), we tested for isolation-by-distance (IBD), pairwise genetic differentiation  
216 between sub-populations (both  $F_{ST}$  and  $D_{est}$ , Weir & Cockerham 1984; Jost 2008), and

217 conducted individual-based spatial Bayesian clustering analyses. IBD was tested with a  
218 permutation test of the relationship between the Euclidean geographic distance matrix (both  
219 untransformed distances in kilometers and logarithm transformed distances) and the pairwise  
220 relatedness matrix ( $F_{ij}$ ; Ritland 1996) with 5000 randomizations using SPAGeDi 1.4 (Hardy  
221 & Vekemans 2002). To visualize the relationship, the pairwise  $F_{ij}$ s were divided into distance  
222 classes with approximately equal numbers of comparisons and averaged to acquire a distance  
223 class specific  $F_{(d)}$ . We estimated the pairwise differentiation coefficients and their 95%  
224 confidence intervals using the R package “diveRsity” (Keenan *et al.* 2013) with 5000  
225 bootstraps for both estimates and for both species.

226 The individual based Bayesian clustering was conducted in the program TESS 2.3.1 (Chen *et*  
227 *al.* 2007; Durand *et al.* 2009). Because all individuals from the same sub-population shared  
228 the same coordinates, we randomized geographic coordinates for each individual in TESS  
229 2.3.1. Specifically, latitude and longitude were allowed to vary a maximum of one kilometer  
230 from the sampling site coordinates, with a standard deviation of 300 meters. Given what is  
231 known about the dispersal of *Calopteryx* individuals (Stettmer 1996; Schutte *et al.* 1997;  
232 Ward & Mill 2007), we considered these parameters for the randomization to be biologically  
233 realistic. Assuming a conditional autoregressive Gaussian admixture model with a quadratic  
234 trend surface degree, we ran TESS 2.3.1 for 50 000 iterations (preceded by a 50 000 iteration  
235 burn-in) 20 times for each maximum number of clusters ( $K_{max}$ ), which ranged from two to the  
236 number of sampling localities ( $K_{max}= 2\dots 18$ , and  $K_{max}= 2\dots 40$ , for *C. splendens* and *C. virgo*,  
237 respectively). We used deviance information criterion (*DIC*) values and stabilization of the *Q*-  
238 matrix of posterior probabilities (i.e. when the number of observed clusters did not increase  
239 with an increase in  $K_{max}$ ) as metrics for choosing the most appropriate  $K_{max}$  for the data. An

240 admixture model was assumed since such models are expected to be more robust against  
241 overestimation of  $K_{max}$  when there are genetic clines (Guillot 2009; François & Durand 2010).  
242 Once  $K_{max}$  was deduced, 80 additional replicate runs were conducted to yield a total of 100  
243 replicate runs for  $K_{max}$ . From these, 20 runs with the lowest  $DIC$  values were used to calculate  
244 average individual admixture proportions with CLUMPP 1.1.2 (Jakobsson & Rosenberg  
245 2007). These were visualized as a bar plot using DISTRUCT 1.1 (Rosenberg 2004).

246 To study how genetic differentiation is related to the locality characteristics and density of  
247 heterospecific *Calopteryx* males, we used a Bayesian population based analysis of genetic  
248 differentiation in the program GESTE2 (Foll & Gaggiotti 2006). GESTE2 incorporates non-  
249 genetic information in the prior distribution of population specific  $F_{ST}$ -values via a  
250 generalized linear model (Balding 2003; Foll & Gaggiotti 2006). GESTE2 analyses were run  
251 for a total of 100 000 iterations with a 50 000 iteration burn-in period.

252 Since the sizes of the two datasets differed both in the amount of genetic information derived  
253 from each individual (12 loci vs. 6 loci for *C. splendens* and *C. virgo*, respectively) and in  
254 their geographic scope (18 sub-populations spanning c.a. 270km vs. 40 sub-populations  
255 spanning c.a. 700km for *C. splendens* and *C. virgo*, respectively), subsets of the data were  
256 analyzed to confirm the results obtained from the total dataset. To do this, we conducted  
257 clustering in TESS for five subsets of the *C. splendens* dataset: three subsets each with six  
258 randomly selected loci, one subset with the same loci that were used in the *C. virgo* dataset,  
259 and one subset excluding the loci that showed some deviations from the assumptions (see  
260 above; Supplementary material F, Fig. F1) In addition, a subset of the *C. virgo* dataset  
261 including only the sympatric populations was analyzed. This was done for two reasons: (1) to  
262 check if possible differences in the SGSs of the two *Calopteryx* species are associated with

263 the different geographic scales of sampling and different numbers of sampled sub-  
264 populations, and (2) to account for the negative spatial autocorrelation in the density of *C.*  
265 *splendens* males (see above).

## 266 RESULTS

267 The genetic diversities of the two species were related to different locality characteristics. The  
268  $uH_E$  of *C. splendens* sub-populations was related to potential connectivity, with increasing  
269 potential connectivity being coupled with increasing  $uH_E$  (Table 1). Interestingly, *C.*  
270 *splendens*  $uH_E$  was differentially related to the densities of conspecific versus heterospecific  
271 males: increasing density of conspecific males was coupled with increasing  $uH_E$ , whereas the  
272 increasing density of heterospecific males was coupled with decreasing  $uH_E$ . Surprisingly,  
273 none of the locality characteristics were related to the  $AR$  of *C. splendens*. The genetic  
274 diversity of *C. virgo* (both  $uH_E$  and  $AR$ ) was related to latitude and longitude, with genetic  
275 diversity increasing with decreasing latitude and increasing longitude (Table 1).

276 There was no statistically significant IBD pattern in pairwise individual kinship coefficients  
277 ( $F_{ij}$ ) of *C. splendens* (Fig 2a; jackknifed  $\beta = -6.32 \times 10^{-5}$ , S.E. =  $1.34 \times 10^{-5}$ ; 95% CI of permuted  
278 null distribution =  $[-6.67 \times 10^{-5}, -3.55 \times 10^{-5}]$ ;  $P = 0.129$ ), although there was a distinct decrease  
279 in the kinship coefficients after the first distance class. On the other hand, for *C. virgo*,  
280 kinship coefficients decreased gradually from the first distance class onwards, and a  
281 statistically significant IBD pattern was observed (Fig 2b;  $\beta = -7.89 \times 10^{-6}$ , S.E. =  $3.16 \times 10^{-6}$ ; 95%  
282 CI of permuted null distribution =  $[-2.31 \times 10^{-6}, -1.62 \times 10^{-7}]$ ;  $P < 0.001$ ). However, although a  
283 statistically significant IBD is observed, it must be noted that the  $F_{ij}$  values for *C. virgo* are  
284 very low.

285 Furthermore, different levels of genetic differentiation were observed in the two species, both  
286 in comparisons of pairwise genetic differentiation and in individual based clustering analyses.  
287 For most of the pairwise comparisons between the *C. splendens* sub-populations, the lower  
288 boundary of the 95% confidence interval was well above 0.01 for both  $F_{ST}$  and  $D_{est}$   
289 (Supplementary material D, Tables D1 and D2), whereas for majority of the pairwise  
290 comparisons between *C. virgo* sub-populations, the 95% confidence intervals either included,  
291 or were very close to zero at the lower boundaries (Supplementary material D, Tables D3 and  
292 D4). Moreover, the individual based genetic clustering in TESS resulted in a different  $K_{max}$  for  
293 the two species. For *C. splendens* six genetic clusters were detected, whereas for *C. virgo* only  
294 two clusters were detected (Fig. 3). Five of the six *C. splendens* clusters each had a high  
295 affinity with a single sub-population, and the remaining cluster had a high affinity with five  
296 sub-populations in central Finland (Fig. 3a). In *C. virgo* the two genetic clusters seemed to  
297 represent two ends of a cline, with one cluster having a high affinity with nine sub-  
298 populations from southern and eastern Finland and the other with ten sub-populations in  
299 northern Finland. Sub-populations geographically intermediate to those in the two clusters  
300 showed different levels of admixture (Fig. 3b).

301 In addition to differences in the number of genetic clusters observed for the two *Calopteryx*  
302 species, differentiation of the sub-populations was related to different locality characteristics  
303 (Tables 1, 2 & 3; Fig. 4). In the GESTE2 analysis of *C. splendens* population specific  $F_{STs}$ ,  
304 the models with potential connectivity were assigned higher posterior probabilities than most  
305 other models (Table 2) resulting in a summed posterior probability of 0.605 for potential  
306 connectivity. Of all the models, a model with constant and potential connectivity received the  
307 highest posterior probability, indicating that population specific  $F_{ST}$  decreases with increasing



308 potential connectivity (Table 3; Fig. 4a). For *C. virgo*, however, latitude received the highest  
309 summed posterior probability (0.994) and a model with constant and latitude had the highest  
310 posterior probability, indicating that  $F_{ST}$  increases with increasing latitude (Table 3; Fig. 4b).  
311 The population specific  $F_{ST}$ s of the *C. virgo* sub-populations were also lower than those of *C.*  
312 *splendens* sub-populations (Fig. 4).

313 The difference between the two species in the number of observed genetic clusters was most  
314 likely not an artifact of a difference in the number of microsatellite loci analyzed, since all of  
315 the *C. splendens* subsets also detected more than two clusters (observed  $K_{max}$  was 3, 3, 5, 4  
316 and 4 in the five respective subsets; Supplementary material F, Fig. F1). Also, the IBD pattern  
317 observed in the SGS of *C. virgo* remained statistically significant even when the dataset was  
318 reduced to a subset including only the sub-populations that are sympatric with *C. splendens*  
319 ( $\beta = -7.16 \times 10^{-6}$ , S.E. =  $3.49 \times 10^{-6}$ ; 95% CI of permuted null distribution =  $[-5.65 \times 10^{-6}, 3.98 \times 10^{-7}]$ ;  
320  $P = 0.004$ ). However, other patterns observed from the *C. virgo* dataset disappeared when  
321 the subset of sympatric sub-populations was analyzed: null models were selected for  
322 population specific  $F_{ST}$ ,  $uH_E$  and  $AR$  (Supplementary material F, Tables F1 and F2).

323 *DISCUSSION*

324 Despite the ecological similarities of the sympatric *C. splendens* and *C. virgo* in Finland, they  
325 exhibited markedly different spatial genetic structures (SGS). Rather than supporting the  
326 hypothesis that sympatric species with similar ecologies exhibit similar SGS (Whiteley *et al.*  
327 2006; Dawson 2012; Hughes *et al.* 2013), our results highlight the possibility that ecological  
328 similarity can, in fact, create differences in the SGS of interacting species.

329 *C. splendens*

330 The positive relationship between unbiased expected heterozygosity ( $uH_E$ ) and density of  
331 conspecific males (Table 1) together with multiple genetic clusters, most corresponding to  
332 individual sub-populations (Fig. 3), indicate predominant effects of drift and low gene flow  
333 on SGS in *C. splendens*. Nonetheless, some legacy of previous gene flow is apparent in the  
334 positive relationship between potential connectivity (i.e. total length of streams within a 5 km  
335 buffer zone) and  $uH_E$  and the negative relationship between potential connectivity and  
336 population specific  $F_{ST}$  (Tables 1 & 3; Fig. 4a). *C. splendens* apparently rarely disperse more  
337 than few kilometers and prefer dispersal along streams (Stettmer 1996; Schutte *et al.* 1997;  
338 Ward & Mill 2007; Chaput-Bardy *et al.* 2008). Previous studies have also found dispersing *C.*  
339 *splendens* to have lower mating success than residents, which can further inhibit gene flow  
340 and strengthen differentiation between *C. splendens* sub-populations (Svensson *et al.* 2006;  
341 Wellenreuther *et al.* 2010).

342 In contrast with previous population genetic studies on this species (Svensson *et al.* 2004;  
343 Chaput-Bardy *et al.* 2008; Viitaniemi 2009) we did not observe IBD (Fig. 2). The absence of

344 IBD could be attributed to genetic disequilibrium due to recent colonization followed by rapid  
345 range expansion (e.g. as seen in *Erythromma viridulum*, Watts *et al.* 2010; and *Coenagrion*  
346 *sticulum*, Swaegers *et al.* 2013) or strong overall differentiation due to range-margin effects  
347 (Eckert *et al.* 2008). Regarding recent colonization, no major shifts in the species range in  
348 Finland have been recorded in recent decades. Although post-glacial colonization can still be  
349 reflected in the SGS, it remains unclear if short-term effects (i.e. within the range of decades;  
350 Watts *et al.* 2010; Swaegers *et al.* 2013) influence the SGS of *C. splendens*. Regarding range-  
351 margin effects, the overall genetic differentiation of *C. splendens* observed here did not differ  
352 greatly from that observed in different regions (overall  $F_{ST}=0.11$  vs. 0.05 in southern Sweden  
353 and 0.14 in western France; Svensson *et al.* 2004; Chaput-Bardy *et al.* 2008). Although our  
354 results cannot be directly compared to those of previous studies because different types of  
355 markers with different levels of polymorphism were used (microsatellites vs. AFLPs;  
356 Jakobsson *et al.* 2013), similar overall differentiation between sub-populations in the Finnish  
357 *C. splendens* population were also found using AFLPs ( $\Phi_{PT}=0.08$ ; Viitaniemi 2009). Most  
358 likely, the lack of IBD is due to strong genetic differentiation between some neighboring sub-  
359 populations (e.g. pairwise  $F_{ST}=0.20$  between sub-populations AK & CJ, Fig. 1;  
360 Supplementary material D, Table D1), as a result of our sampling, which focused on sub-  
361 populations at different rivers separated by land and/or lakes.

362 An interesting finding in our analysis is that interspecific interactions could contribute to the  
363 strength of drift experienced by *C. splendens* sub-populations (Table 1): when *C. virgo* males  
364 are abundant, sympatric *C. splendens* sub-populations are less genetically diverse.  
365 Alternatively, such a relationship could arise due to habitat partitioning and local adaptation  
366 of the two species to different habitats. In Central Europe, the two *Calopteryx* species do

367 show different preferences to water temperature (Sternberg & Buchwald 1999; Schütte &  
368 Schrimpf 2002). If locally adapted, the population sizes of the two species would be expected  
369 to show opposing trends with water temperature. However, our data show that in Finland the  
370 preferred water temperatures of the two species overlap. Although we have not quantified  
371 habitat partitioning with respect to other environmental variables, densities of the two species  
372 in sympatric sub-populations do not correlate negatively, suggesting that possible habitat  
373 partitioning between the species is not strong (Supplementary material A).

374 Our results are concordant with a study by Viitaniemi (2009), who suggested that interspecific  
375 interactions explained a decline in the heterozygosity of *C. splendens* with increasing  
376 proportion of *C. virgo* individuals in sympatric sub-populations. We believe that the density  
377 of *C. virgo*, rather than its relative abundance in sympatric populations, better describes how  
378 frequently heterospecific individuals are encountered and is a more appropriate measure of  
379 the frequency of interspecific interactions. Unfortunately, with our data it is not possible to  
380 determine how interspecific interactions shape the effective population sizes of sub-  
381 populations directly, or reduce gene flow, or both (e.g. as suggested by Ranta *et al.* 2009). It  
382 is known that increasing aggression of *C. virgo* males towards *C. splendens* males decreases  
383 the proportion of *C. splendens* males with a territorial mating strategy in Finnish sub-  
384 populations (Tynkkynen *et al.* 2004, 2006). Since territorial males have better mating success  
385 than non-territorial males (Plaistow & Siva-Jothy 1996), it is likely that *C. virgo* influences  
386 the effective population size by accentuating the reproductive skew of *C. splendens*.  
387 However, it is also possible that interspecific aggression limits gene flow by reducing either  
388 the immigration of *C. splendens* (i.e. males immigrate to sites with fewer *C. virgo*), or the  
389 probability that immigrants obtain matings (i.e. immigrant males are not able to hold

390 territories at sites with high *C. virgo* abundance). Future work should examine the role of  
391 interspecific aggression as a likely explanation for the observed negative relationship between  
392 *C. splendens* genetic diversity and *C. virgo* density.

393 Although  $uH_E$  of *C. splendens* was negatively related to *C. virgo* density, the effect of *C.*  
394 *virgo* density was not identified in the analysis of population specific  $F_{ST}$ s of *C. splendens*,  
395 which could be due to the low statistical power of GESTE2 (Balkenhol *et al.* 2009). It is more  
396 puzzling, however, that we did not observe similar locality-specific patterns with  $AR$  as we  
397 did with  $uH_E$  (Table 1, Supplementary material E, Table E1), since allelic richness and  
398 heterozygosity often exhibit concordant patterns even though allelic richness is more sensitive  
399 to changes in effective population size (Luikart *et al.* 1998; Eckert *et al.* 2008). A reasonable  
400 explanation for this counterintuitive pattern could be that allelic richness of the Finnish *C.*  
401 *splendens* population is already low, and most variation in  $AR$  between sub-populations is in  
402 the relative frequencies of common alleles (Supplementary material C, Table C3). Indeed, the  
403 average number of alleles per locus observed across the whole *C. splendens* dataset is 3.38  
404 (S.D. = 1.59) and the average frequency of the most common allele is 0.72 (S.D. = 0.20),  
405 leading to a situation in which the relative amount of variation is higher in  $uH_E$  than in  $AR$   
406 (Coefficient of variation = 0.130 and 0.086 respectively). This could be a consequence of the  
407 markers, since variability of microsatellite loci in many dragonflies and damselflies is  
408 generally low (Watts 2009), or alternatively reflect the fact that populations close to range-  
409 margins generally have low genetic diversities (Eckert *et al.* 2008; Watts *et al.* 2010).

410 *C. virgo*

411 To our knowledge the SGS of *C. virgo* has not been described before, and to our surprise, we  
412 observed an SGS that was very different from that observed in *C. splendens*. Latitude was  
413 consistently related to all aspects of the SGS of *C. virgo* ( $uH_E$ ,  $AR$ , IBD, clustering and  
414 population specific  $F_{ST}$ , Tables 1, 2 & 3; Figures 2b, 3b, and 4b). In addition to latitude, the  
415 genetic diversity of *C. virgo* was also related to longitude, with increasing longitude being  
416 coupled with increasing genetic diversity (Table 1). The relationship of *C. virgo*'s SGS with  
417 latitude and longitude probably reflects a persistent signature of colonization after the last  
418 glacial maximum, and latitudinal gradients in genetic diversity at geographic scales similar to  
419 ours have been reported before (Hewitt 2000; Schmitt & Seitz 2001). Alternatively, the  
420 latitudinal trends in diversity and differentiation can result from range margin effects, which  
421 are difficult to differentiate from the effects of post glacial colonization (Eckert *et al.* 2008).  
422 On the other hand, low genetic differentiation between sub-populations and a latitudinal cline  
423 in genetic diversity could be due genetic disequilibrium caused by recent colonization or  
424 range expansion (Watts *et al.* 2010; Swaegers *et al.* 2013); but, similarly to the case of *C.*  
425 *splendens*, we cannot determine this since we do not have adequate temporal data on changes  
426 in SGS or know the timing of colonization of Finland by *C. virgo*. However, although two  
427 genetic clusters are discovered and some pairwise genetic differences between southern and  
428 northern *C. virgo* sub-populations are significant (Supplementary material D, Tables D3 and  
429 D4) the genetic differentiation is low, which likely reflects gene flow between populations.  
430 Indeed, this is supported by the cline of admixture between the genetic clusters and low  
431 population specific  $F_{ST}$ s (Figures 3b & 4b).

432 *Why is there a difference between the SGSs of the two Calopteryx species?*

433 Although it is not unprecedented that closely related species show different SGSs (Hodges *et*  
434 *al.* 2007; Lehrian *et al.* 2009; Fortuna *et al.* 2009; Johansson *et al.* 2013), our results are  
435 surprising considering the similarities in the life-history characteristics of the two species and  
436 extent of habitat overlap in Fennoscandia. The different SGSs of the two *Calopteryx* species  
437 are unlikely to be explained by statistical artifacts, such as differences in the information  
438 content of the loci (Supplementary material C, Tables C2, C3 and C4; Jakobsson *et al.* 2013),  
439 different numbers of sampled loci, or different geographic ranges of sampling (Supplementary  
440 material F). However, since latitudinal and longitudinal variation were not significantly  
441 related to the SGS of *C. virgo* in a subset consisting only of the sympatric sub-populations, we  
442 cannot rule out the possibility that the lack of relationship between latitude and longitude with  
443 *C. splendens* SGS is partly due to a difference in scale.

444 Our study shows that the SGS of *C. splendens* is influenced more by genetic drift than is the  
445 SGS of *C. virgo*. Furthermore, our results suggest that interspecific interactions between *C.*  
446 *splendens* and *C. virgo* contribute to this difference by strengthening the magnitude of genetic  
447 drift experienced by *C. splendens* sub-populations while the SGS of *C. virgo* is not affected.  
448 A likely explanation for this discrepancy is that, in Finland, *C. virgo* is the stronger  
449 competitor for the best breeding habitats (Tynkkynen *et al.* 2004, 2005, 2006). The  
450 competitive advantage of *C. virgo* could be due to its ability to occupy a wider thermal niche  
451 in Fennoscandia (Wellenreuther *et al.* 2012; Supplementary material A). Indeed, *C. virgo* is  
452 more common in Finland than *C. splendens* and this seems to be also reflected in its SGS: the  
453 abundance of sub-populations offer more stepping stones for dispersal and gene flow, leading  
454 to less genetic differentiation between *C. virgo* sub-populations.

455 *Conservation implications*

456 Since characterizing SGS of multiple species for landscape level conservation planning is  
457 often unfeasible, surrogate approaches are in high demand from practicing conservationists to  
458 aid prediction of the effects of different management and land-use actions (Margules &  
459 Pressey 2000; Rodrigues & Brooks 2007). Although neither *C. splendens* nor *C. virgo* is  
460 endangered, the two species system can function as a test of the surrogate approach. Our  
461 results highlight the fact that use of a surrogate requires careful consideration. Interspecific  
462 interactions are an important part of the community that can have a role in shaping the SGS of  
463 natural populations, and should not be overlooked in conservation planning.

464 *ACKNOWLEDGEMENTS*

465 We are grateful to four anonymous referees, Matti Häkkinen, Reetta Hänninen, Sami  
466 Karjalainen, Matti Koivula, Maria Marjeta, Adriano Mazziotta, Kaisa Mustola, Mikael  
467 Puurtinen, Jenni Tikka, Janne Valkonen and the members of MCC for constructive feedback  
468 and helping out with various aspects of the manuscript.

469 This research was funded by the Academy of Finland (via Biological Interactions Graduate  
470 School, The Center of Excellence for Evolutionary Research, and the postdoctoral project  
471 funding of Katja Kuitunen), *Kone Foundation*, The Finnish Cultural Fund, Kuopion  
472 Luonnonystävään Yhdistys, and Societas Biologica Fennica Vanamo.



## 473 LITERATURE

- 474 Askew RR (2004) *The Dragonflies of Europe*. Harley Books, Colchester.
- 475 Balding DJ (2003) Likelihood-based inference for genetic correlation coefficients.  
476 *Theoretical Population Biology*, **63**, 221–230.
- 477 Balkenhol N, Waits LP, Dezzani RJ (2009) Statistical approaches in landscape genetics: an  
478 evaluation of methods for linking landscape and genetic data. *Ecography*, **32**, 818–830.
- 479 Bohonak AJ (1999) Dispersal, gene flow, and population structure. *The Quarterly Review of*  
480 *Biology*, **74**, 21–45.
- 481 Calabrese JM, Fagan WF (2004) A comparison-shopper's guide to connectivity metrics.  
482 *Frontiers in Ecology and the Environment*, **2**, 529–536.
- 483 Chaput-Bardy A, Lemaire C, Picard D, Secondi J (2008) In-stream and overland dispersal  
484 across a river network influences gene flow in a freshwater insect, *Calopteryx splendens*.  
485 *Molecular Ecology*, **17**, 3496–3505.
- 486 Chen C, Durand E, Forbes F, François O (2007) Bayesian clustering algorithms ascertaining  
487 spatial population structure: a new computer program and a comparison study.  
488 *Molecular Ecology Notes*, **7**, 747–756.
- 489 Corbet PS (1999) *Dragonflies: Behaviour and Ecology of Odonata*. Harley Books, Essex.
- 490 Cribari-Neto F, Zeileis A (2010) Beta Regression in R. *Journal of Statistical Software*, **34**, 1–  
491 24.
- 492 Dawson MN (2012) Parallel phylogeographic structure in ecologically similar sympatric  
493 sister taxa. *Molecular Ecology*, **21**, 987–1004.
- 494 Durand E, Jay F, Gaggiotti OE, François O (2009) Spatial inference of admixture proportions  
495 and secondary contact zones. *Molecular Biology and Evolution*, **26**, 1963–1973.
- 496 Eckert CG, Samis KE, Loughheed SC (2008) Genetic variation across species' geographical  
497 ranges: the central-marginal hypothesis and beyond. *Molecular Ecology*, **17**, 1170–1188.
- 498 Foll M, Gaggiotti O (2006) Identifying the environmental factors that determine the genetic  
499 structure of populations. *Genetics*, **174**, 875–891.
- 500 Fortuna MA, Albaladejo RG, Fernández L, Aparicio A, Bascompte J (2009) Networks of  
501 spatial genetic variation across species. *Proceedings of the National Academy of*  
502 *Sciences of the United States of America*, **106**, 19044–19049.
- 503 François O, Durand E (2010) Spatially explicit Bayesian clustering models in population  
504 genetics. *Molecular Ecology Resources*, **10**, 773–784.

- 505 Frankham R (2005) Genetics and extinction. *Biological Conservation*, **126**, 131–140.
- 506 Funk DJ, Egan SP, Nosil P (2011) Isolation by adaptation in *Neochlamisus* leaf beetles: host-  
507 related selection promotes neutral genomic divergence. *Molecular Ecology*, **20**, 4671–  
508 4682.
- 509 Goudet J (1995) FSTAT (Version 1.2): A Computer Program to Calculate F-Statistics.  
510 *Journal of Heredity*, **86**, 485–486.
- 511 Guillot G (2009) On the inference of spatial structure from population genetics data.  
512 *Bioinformatics*, **25**, 1796–1801.
- 513 Hardy OJ, Vekemans X (2002) SPAGeDi: a versatile computer program to analyse spatial  
514 genetic structure at the individual or population levels. *Molecular Ecology*, **2**, 618–620.
- 515 Hewitt G (2000) The genetic legacy of the Quaternary ice ages. *Nature*, **405**, 907–913.
- 516 Hodges KM, Rowell DM, Keogh JS (2007) Remarkably different phylogeographic structure  
517 in two closely related lizard species in a zone of sympatry in south-eastern Australia.  
518 *Journal of Zoology*, **272**, 64–72.
- 519 Honkavaara J, Dunn DW, Ilvonen S, Suhonen J (2011) Sympatric shift in a male sexual  
520 ornament in the damselfly *Calopteryx splendens*. *Journal of Evolutionary Biology*, **24**,  
521 139–145.
- 522 Hughes JM, Huey JA, Schmidt DJ (2013) Is realised connectivity among populations of  
523 aquatic fauna predictable from potential connectivity? *Freshwater Biology*, **58**, 951–966.
- 524 Ilvonen S, Ilvonen JJ, Kaunisto KM, Krams I, Suhonen J (2011) Can infection by eugregarine  
525 parasites mediate species coexistence in *Calopteryx* damselflies? *Ecological*  
526 *Entomology*, **36**, 582–587.
- 527 Jakobsson M, Edge MD, Rosenberg NA (2013) The relationship between F(ST) and the  
528 frequency of the most frequent allele. *Genetics*, **193**, 515–528.
- 529 Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program  
530 for dealing with label switching and multimodality in analysis of population structure.  
531 *Bioinformatics*, **23**, 1801–1806.
- 532 Johansson H, Stoks R, Nilsson-Örtman V, Ingvarsson PK, Johansson F (2013) Large-scale  
533 patterns in genetic variation, gene flow and differentiation in five species of European  
534 Coenagrionid damselfly provide mixed support for the central-marginal hypothesis.  
535 *Ecography*, **36**, 744–755.
- 536 Jost L (2008)  $G_{ST}$  and its relatives do not measure differentiation. *Molecular Ecology*, **17**,  
537 4015–4026.
- 538 Karjalainen S (2010) *Suomen sudenkorennot [The Dragonflies of Finland]*. Tammi, Helsinki.

- 539 Karjalainen S, Hämäläinen M (2013) *Neidonkorennot - Demoiselle Damselflies*. Caloptera  
540 Publishing, Helsinki.
- 541 Keenan K, McGinnity P, Cross TF, Crozier WW, Prodöhl PA (2013) diveRsity: an R package  
542 for the estimation and exploration of population genetics parameters and their associated  
543 errors. *Methods in Ecology and Evolution*, **4**, 782–788.
- 544 Keränen I, Kahilainen A, Knott KE, Kotiaho JS, Kuitunen K (2013) High maternal species  
545 density mediates unidirectional heterospecific matings in *Calopteryx* damselflies.  
546 *Biological Journal of the Linnean Society*, **108**, 534–545.
- 547 Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite  
548 population. *Genetics*, **49**, 725–738.
- 549 Kuitunen K, Kotiaho JS, Luojumäki M, Suhonen J (2011) Selection on size and secondary  
550 sexual characters of the damselfly *Calopteryx splendens* when sympatric with the  
551 congener *Calopteryx virgo*. *Canadian Journal of Zoology*, **89**, 1–9.
- 552 Lehrian S, Pauls SU, Haase P (2009) Contrasting patterns of population structure in the  
553 montane caddisflies *Hydropsyche tenuis* and *Drusus discolor* in the Central European  
554 highlands. *Freshwater Biology*, **54**, 283–295.
- 555 Lessells CM, Boag PT (1987) Unrepeatable Repeatabilities: A Common Mistake. *The Auk*,  
556 **104**, 116–121.
- 557 Luikart G, Sherwin WB, Steele BM, Allendorf FW (1998) Usefulness of molecular markers  
558 for detecting population bottlenecks via monitoring genetic change. *Molecular Ecology*,  
559 **7**, 963–974.
- 560 De Marchi G (1990) Precopulatory reproductive isolation and wing colour dimorphism in  
561 *Calopteryx splendens* females in southern Italy (Zygoptera: Calopterygidae).  
562 *Odonatologica*, **19**, 243–250.
- 563 Margules CR, Pressey RL (2000) Systematic Conservation Planning. *Nature*, **405**, 243–253.
- 564 Molecular Ecology Resources Primer Development Consortium *et al.* (2011) Permanent  
565 genetic resources added to molecular ecology resources database 1 February 2011–31  
566 March 2011. *Molecular Ecology Resources*, **11**, 757–758.
- 567 Nestmann S, Sretenovic Rajcic T, Dehmer KJ, Fischer M, Schumacher J, Roscher C (2011)  
568 Plant species diversity and composition of experimental grasslands affect genetic  
569 differentiation of *Lolium perenne* populations. *Molecular Ecology*, **20**, 2188–2203.
- 570 Outomuro D, Ocharan FJ (2011) Wing pigmentation in *Calopteryx* damselflies: a role in  
571 thermoregulation? *Biological Journal of the Linnean Society*, **103**, 36–44.
- 572 Peakall R, Smouse P (2012) GenAlEx 6.5: Genetic analysis in Excel. Population genetic  
573 software for teaching and research – an update. *Bioinformatics*, **1**, 6–8.

- 574 Plaistow S, Siva-Jothy MT (1996) Energetic constraints and male mate-securing tactics in the  
575 damselfly *Calopteryx splendens xanthostoma* (Chapentier). *Proceedings of the Royal*  
576 *Society of London, Series B: Biological Sciences*, **263**, 1233–1238.
- 577 R Core Team (2012) R: A Language and Environment for Statistical Computing.
- 578 Rangel TF, Diniz-Filho JAF, Bini LM (2010) SAM: a comprehensive application for Spatial  
579 Analysis in Macroecology. *Ecography*, **33**, 46–50.
- 580 Ranta E, Bach LA, Kaitala V, Björklund M, Lundberg P (2009) Competition enhances spatial  
581 genetic differentiation. *Evolutionary Ecology Research*, **11**, 1251–1258.
- 582 Ritland K (1996) Estimators for pairwise relatedness and individual inbreeding coefficients.  
583 *Genetical Research Cambridge*, **67**, 175–185.
- 584 Rodrigues ASL, Brooks TM (2007) Shortcuts for Biodiversity Conservation Planning: the  
585 Effectiveness of Surrogates. *Annual Review of Ecology, Evolution and Systematics*, **38**,  
586 713–737.
- 587 Rosenberg NA (2004) Distruct: a program for the graphical display of population structure.  
588 *Molecular Ecology Notes*, **4**, 137–138.
- 589 Schmitt T, Seitz A (2001) Allozyme variation in *Polyommatus coridon* (Lepidoptera:  
590 Lycaenidae): identification of ice-age refugia and reconstruction of post-glacial  
591 expansion. *Journal of Biogeography*, **28**, 1129–1136.
- 592 Schutte G, Reich M, Plachter H (1997) Mobility fo the relobiont damselfly *Calopteryx*  
593 *splendens* (Harris) in fragmented habitats (Zygoptera: Calopterygidae). *Odonatologica*,  
594 **26**, 317–327.
- 595 Schütte C, Schrimpf I (2002) Explaining species distribution in running water systems: larval  
596 respiration and growth of two *Calopteryx* species (Odonata, Zygoptera). *Archiv Fur*  
597 *Hydrobiologie*, **153**, 217–229.
- 598 Slatkin M (1985) Gene Flow in Natural Populations. *Annual Review of Ecology and*  
599 *Systematics*, **16**, 393–430.
- 600 Sternberg K, Buchwald R (1999) Einnischung von *Calopteryx virgo* und *Calopteryx*  
601 *splendens*. In: *Die Libellen Bade-Württembergs Bd. 1*, pp. 211–212. Eugen Ulmer,  
602 Stuttgart, Germany.
- 603 Stettmer C (1996) Colonisation and dispersal patterns of banded (*Calopteryx splendens*) and  
604 beautiful demoiselles (*C. virgo*) (Odonata: Calopterygidae) in south-east German  
605 streams. *European Journal of Entomolgy*, **93**, 579–593.
- 606 Svensson EI, Eroukhmanoff F, Friberg M (2006) Effects of natural and sexual selection on  
607 adaptive population divergence and premating isolation in a damselfly. *Evolution*, **60**,  
608 1242–1253.

- 609 Svensson EI, Karlsson K, Friberg M, Eroukhmanoff F (2007) Gender differences in species  
610 recognition and the evolution of asymmetric sexual isolation. *Current Biology*, **17**, 1943–  
611 1947.
- 612 Svensson EI, Kristoffersen L, Oskarsson K, Bensch S (2004) Molecular population  
613 divergence and sexual selection on morphology in the banded demoiselle (*Calopteryx*  
614 *splendens*). *Heredity*, **93**, 423–433.
- 615 Svensson EI, Waller JT (2013) Ecology and sexual selection: evolution of wing pigmentation  
616 in Calopterygid damselflies in relation to latitude, sexual dimorphism, and speciation.  
617 *The American Naturalist*, **182**, E174–E195.
- 618 Swaegers J, Mergeay J, Therry L, Larmuseau MHD, Bonte D, Stoks R (2013) Rapid range  
619 expansion increases genetic differentiation while causing limited reduction in genetic  
620 diversity in a damselfly. *Heredity*, **111**, 422–429.
- 621 Thompson DJ, Hassall C, Lowe CD, Watts PC (2011) Field estimates of reproductive success  
622 in a model insect: behavioural surrogates are poor predictors of fitness. *Ecology Letters*,  
623 **14**, 905–913.
- 624 Tynkkynen K, Kotiaho JS, Luojumäki M, Suhonen J (2005) Interspecific aggression causes  
625 negative selection on sexual characters. *Evolution*, **59**, 1838–1843.
- 626 Tynkkynen K, Kotiaho JS, Luojumäki M, Suhonen J (2006) Interspecific territoriality in  
627 *Calopteryx* damselflies: the role of secondary sexual characters. *Animal Behaviour*, **71**,  
628 299–306.
- 629 Tynkkynen K, Rantala MJ, Suhonen J (2004) Interspecific aggression and character  
630 displacement in the damselfly *Calopteryx splendens*. *Journal of Evolutionary Biology*,  
631 **17**, 759–767.
- 632 Valle KJ (1952) *Suomen eläimet 7: Sudenkorennot = Odonata*. WSOY, Helsinki.
- 633 Viitaniemi HM (2009) Genetic structure and the effects of interspecific aggression on Finnish  
634 *Calopteryx splendens* populations. University of Turku.
- 635 Ward L, Mill P (2007) Long range movements by individuals as a vehicle for range expansion  
636 in *Calopteryx splendens* (Odonata: Zygoptera). *European Journal of Entomology*, **104**,  
637 195–198.
- 638 Watts PC (2009) Characteristics of microsatellite loci in Odonata. *International Journal of*  
639 *Odonatology*, **12**, 275–286.
- 640 Watts PC, Keat S, Thompson DJ (2010) Patterns of spatial genetic structure and diversity at  
641 the onset of a rapid range expansion: colonisation of the UK by the small red-eyed  
642 damselfly *Erythromma viridulum*. *Biological Invasions*, **12**, 3887–3903.

- 643 Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population  
644 structure. *Evolution*, **38**, 1358–1370.
- 645 Wellenreuther M, Larson KW, Svensson EI (2012) Climatic niche divergence or  
646 conservatism? Environmental niches and range limits in ecologically similar damselflies.  
647 *Ecology*, **93**, 1353–1366.
- 648 Wellenreuther M, Vercken E, Svensson EI (2010) A role for ecology in male mate  
649 discrimination of immigrant females in *Calopteryx* damselflies? *Biological Journal of*  
650 *the Linnean Society*, **100**, 506–518.
- 651 Whiteley AR, Spruell P, Allendorf FW (2006) Can common species provide valuable  
652 information for conservation? *Molecular Ecology*, **15**, 2767–2786.
- 653

654 *DATA ACCESSIBILITY*

655 Genotypes for both *Calopteryx splendens* and *Calopteryx virgo*, accompanied with sub-

656 population specific environmental data uploaded to Dryad (doi:10.5061/dryad.v1ss7).

657 *AUTHOR CONTRIBUTIONS*

658 All the authors (AK, IK, KEK, KK and JSK) contributed to the design of the research;  
659 AK, IK and KK conducted the field work; AK, IK and KEK designed the laboratory  
660 protocol and conducted the laboratory work; AK and KEK conducted the analyses; AK  
661 led the writing process of the manuscript, to which all authors contributed.



## 662 TABLES

663 Table 1. The relationships between locality characteristics and genetic diversity of *Calopteryx*  
 664 species. The results considering  $uH_E$  and  $AR$  represent beta regression and multiple linear  
 665 regression models, respectively. For *C. splendens* only a beta regression model for the  
 666 relationship between  $uH_E$  and locality characteristics is displayed, as  $AR$  showed no  
 667 statistically significant relationship with any of the locality characteristics. Descriptions of  
 668 model selection can be found in Supplementary material E.

Species	Dependent	Independent	Estimate	S.E.	z ( $uH_E$ ); t ( $AR$ )	P
<i>C. splendens</i>	$uH_E$	intercept	-0.884	0.139	-6.345	< 0.001
		$\log_{10}(\text{CsD})$	0.156	0.073	2.131	0.033
		PC	0.011	0.005	2.126	0.033
		$\log_{10}(\text{CvD})$	-0.183	0.052	-3.479	< 0.001
<i>C. virgo</i>	$uH_E$	intercept	1.565	0.560	2.794	0.005
		N	$-3.432 \times 10^{-7}$	$8.436 \times 10^{-8}$	-4.068	< 0.001
		E	$3.474 \times 10^{-7}$	$1.593 \times 10^{-7}$	2.181	0.029
	$AR$	intercept	6.365	0.698	9.120	< 0.001
		N	$-5.720 \times 10^{-7}$	$1.051 \times 10^{-7}$	-5.436	< 0.001
		E	$4.451 \times 10^{-7}$	$2.012 \times 10^{-7}$	2.213	0.033

669 Abbreviations of independent variables of the models:  $\log_{10}(\text{CsD})$  = logarithm transformed density estimates of *C. splendens*  
 670 males; PC = potential connectivity (in kilometers; see text for description);  $\log_{10}(\text{CvD})$  = logarithm transformed density  
 671 estimates of *C. virgo* males; N = latitude in UTM coordinates; E = longitude in UTM coordinates.

672 Table 2. The highest posterior probability models for the relationship between population  
 673 specific  $F_{ST}$  and locality characteristics obtained using GESTE2.

Model #	Probability	Locality characteristics included
<i>C. splendens</i>		
3	0.455	PC
1	0.282	Null
4	0.062	$\log_{10}(\text{CsD})$ , PC
<i>C. virgo</i>		
9	0.522	N
25	0.204	N, E
13	0.100	N, $\log_{10}(\text{CsD})$

674 Abbreviations of independent variables of the models: PC = potential connectivity  
 675 (see text for description); N = latitude in UTM coordinates;  $\log_{10}(\text{CsD})$  =  
 676 logarithm transformed estimates of density of *C. splendens* males; E = longitude  
 677 in UTM coordinates. Null stands for a null-model including only the intercept but  
 678 none of the explanatory variables.

679 Table 3. Coefficients of the models with the highest posterior probabilities for population  
 680 specific  $F_{ST}$  (bold in Table 1).

Species	Estimates	Regression coefficient		HDPI 95%	
		Mean	Mode	LL	UL
<i>C. splendens</i>					
	Constant ( $\alpha_0$ )	-2.14	-2.19	-2.47	-1.81
	PC ( $\alpha_2$ )	-0.393	-0.400	-0.699	-0.085
	Variance ( $\sigma^2$ )	0.336	0.257	0.121	0.616
<i>C. virgo</i>					
	Constant ( $\alpha_0$ )	-4.26	-4.21	-4.62	-3.92
	N ( $\alpha_4$ )	0.628	0.622	0.338	0.929
	Variance ( $\sigma^2$ )	0.347	0.262	0.112	0.658

681 Abbreviations of independent variables of the models: PC = potential connectivity (see text  
 682 for description); N = latitude in UTM coordinates.

## 683 FIGURE CAPTIONS

684 Figure 1. Map of *Calopteryx* sampling localities within Finland. Open symbols represent sites  
685 with both *C. virgo* and *C. splendens* in sympatry and solid symbols represent sites with *C.*  
686 *virgo* in allopatry. Letters indicate the locality codes (see Supplementary material B, Table B1  
687 for more information on the localities). The dashed and dotted lines represent the range limit  
688 of *C. splendens* and *C. virgo*, respectively.

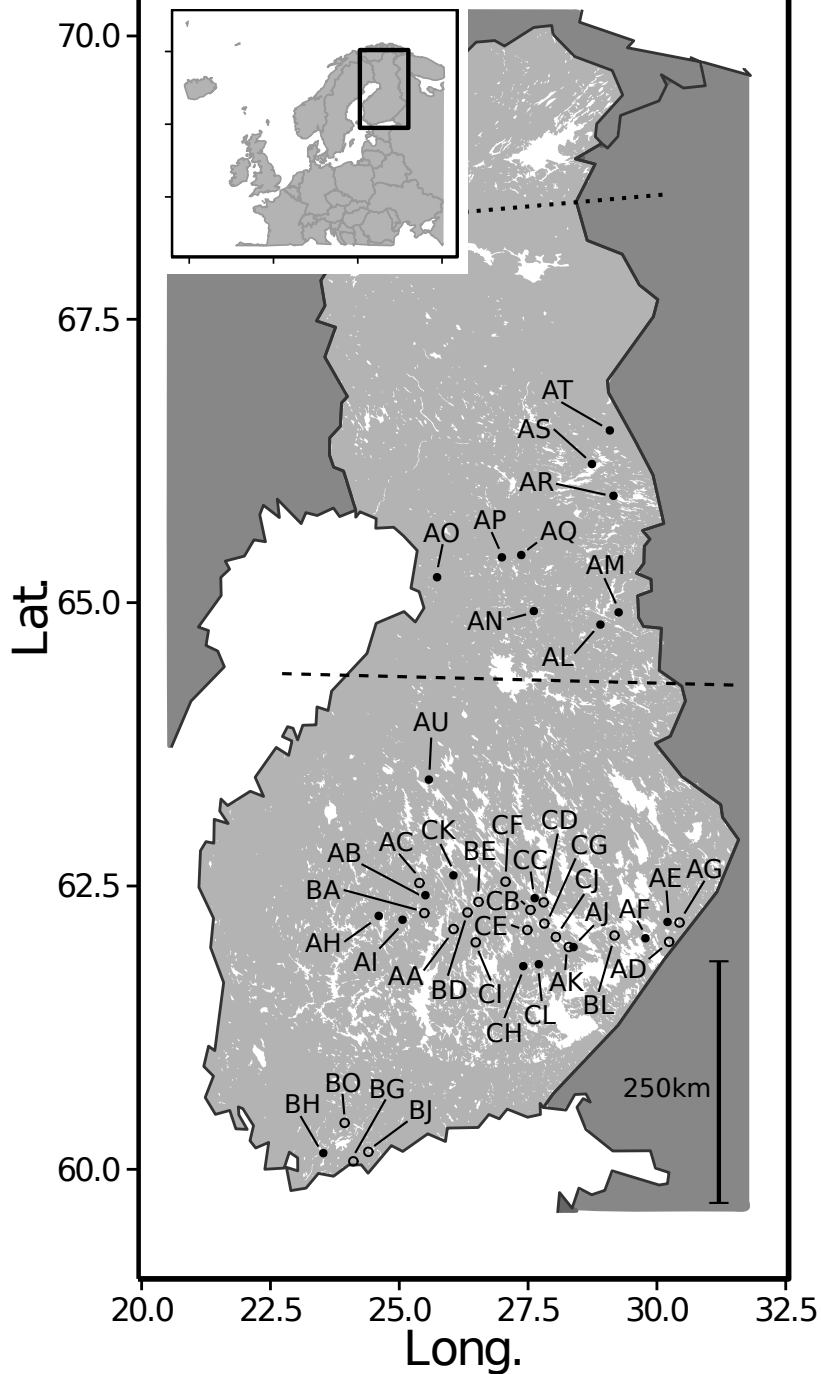
689 Figure 2. A correlogram of average pairwise kinship coefficients ( $F_{(d)}$ ) between individuals of  
690 (a) *C. splendens* and (b) *C. virgo* in different distance classes. The error bars represent  $\pm$  S.E.  
691 for each  $F_{(d)}$ . Note the different scales on the x-axis of the two panels.

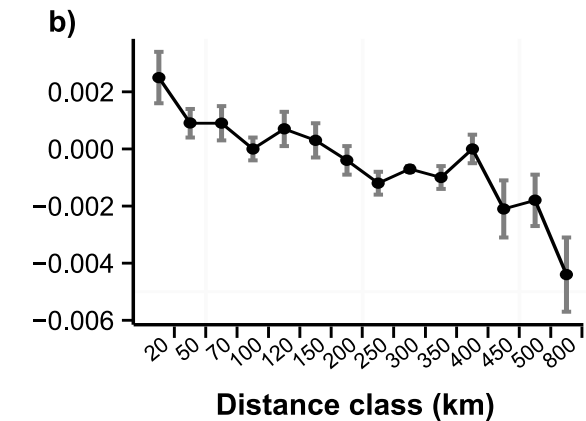
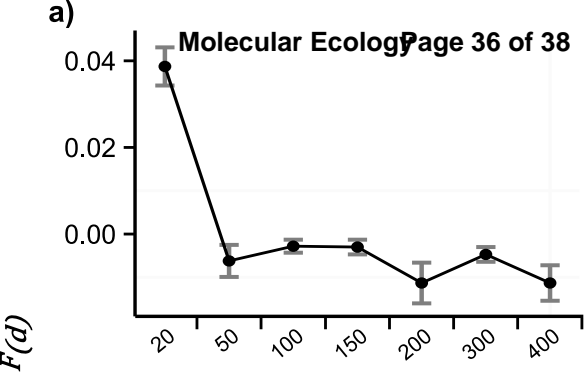
692 Figure 3. The admixture proportions of (a) *C. splendens* and (b) *C. virgo* individuals.  
693 Different colors represent different genetic clusters with  $K=6$  and  $K=2$  for *C. splendens* and *C.*  
694 *virgo*, respectively. The samples in the two panels are arranged according to the geographic  
695 coordinate axis along which the range of variation is the largest. Thus, the samples of (a) *C.*  
696 *splendens* are arranged according to longitude with the westernmost sampling localities on the  
697 left hand side and the samples of (b) *C. virgo* are arranged according to latitude, with  
698 sampling localities at low latitudes on the left hand side. Letters codes indicate the sub-  
699 population (see Supplementary material B, Table B1 for more information on the localities).

700 Figure 4. A graphical representation of the GESTE2 models in Table 3. (a) The relationship  
701 between *C. splendens* population specific  $F_{ST}$  and potential connectivity (i.e. length of rivers  
702 within a five kilometer buffer zone), and (b) the relationship between *C. virgo* population  
703 specific  $F_{ST}$  and latitude (according to UTM grid ETRS-TM35FIN).

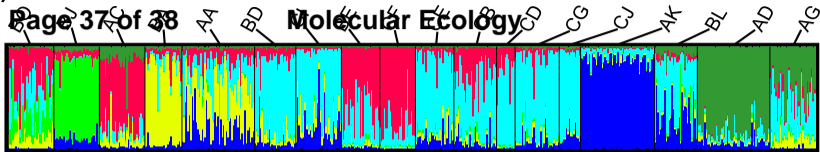
704 *SUPPORTING INFORMATION*

- 705 - Supplementary material A: Overlap in water temperature preferences of *Calopteryx*  
706 species in Finland.
- 707 - Supplementary material B: Characteristics of *Calopteryx* sub-populations and locality  
708 information.
- 709 - Supplementary material C: Genotyping protocols, characteristics of the microsatellite  
710 loci and genetic characteristics of the sub-populations.
- 711 - Supplementary material D: Pairwise genetic differentiation of the *Calopteryx* sub-  
712 populations.
- 713 - Supplementary material E: Model selection for beta regression and linear regression  
714 models on the relationships between locality characteristics and genetic diversity.
- 715 - Supplementary material F: Analyses with subsets of the *Calopteryx* datasets.

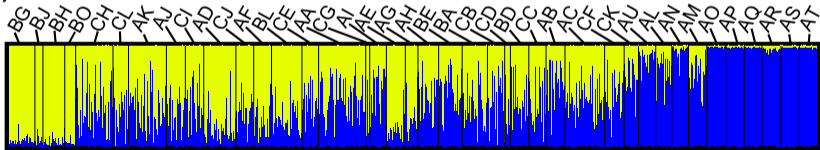




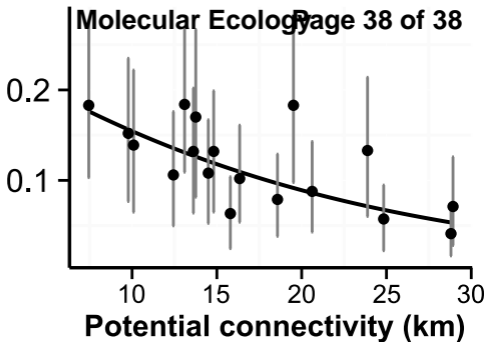
a)



b)



a)



b)

